Peer Review File

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Reviewer A

Comment 1: This manuscript presents a review of the relevant literature relating to the use of anti-VEGF gene therapy in neovascular eye disease. The manuscript looks like an overview of VEGF targeting therapy, rather than a specific focus on gene therapy for neovascular eye diseases. In this respect, the title "Targeting Vascular Endothelial Growth Factor using Retinal Gene Therapy" may appear misleading. Overall, the paper is well written, but it can be further improved by including more details. Also, there are a number of aspects that require attention before acceptance can be considered. Please see the major and minor concerns below.

Response 1: We thank the reviewer for the summary and insight. We were invited to write a review paper on 'Anti-VEGF gene therapy', and since the journal's audience may not already be familiar with ocular anti-angiogenesis therapies, we believe a thorough review of VEGF and current anti-VEGF pharmacotherapies is necessary to provide the context and background to readers. As we noted, most current gene therapies employ a biofactory strategy to mimic current anti-VEGF therapy. The only information that was not relevant to anti-VEGF therapy was retinostat. Thus we have removed it from the main text (please see the comment 11).

Comment 2: Section I "Current anti-angiogenic therapies for retinal diseases"- A summary to point out the pros and cons of different anti-VEGF medicine (as well as laser vs pharmacologic intervention) will help the reader to compare the current clinical available treatments. For anti-VEGF agents, more details, rather than FDA approval story, on the dosing and regimen of recommended administration should be addressed.

Response 2: We have included the use of laser and photodynamic therapies per the reviewer's request, but did not focus on this as they are largely considered second line to pharmacotherapies (lines 83-88). We have also added more details on anti-VEGF dosing and regimen, as well as a summary table of anti-VEGF drugs comparing the difference between drugs, their mechanisms of action, and dosing (Lines 91-157, Table 1).

Comment 3: Lines 108-111: Reference should be provided for the statement "Some reports of an obstructive vasculitis….".

Response 3: The first peer-reviewed report describing this side effect was just published in late April 2020, which we now referenced it in our manuscript (Line 158).

Comment 4: Section II "Emerging anti-angiogenic therapies with increased durability"- I was a bit confused that these approaches h improve the durability of anti-VEGF agents that mentioned in section I or authors thing it can be used to improve current anti-VEGF therapy in the neovascular eye disease? A table may help to summaries the approaches with more detail such as what anti-VEGF agents were used with the strategies, duration, in vivo studies or clinical trials.
Response 4: We have simplified the subheading for Section II to "Emerging anti-angiogenic therapies" to broadly cover methods that increase efficacy or durability, although most of these focus on increasing durability. We have added a table summarizing the difference between these methods (Table 2).

Comment 5: Lines 114-118: The authors could summaries the issues of current anti-VEGF treatment associated with repeat injections, which results in an unfavourable benefit-risk ratio in the treatment of ocular angiogenesis. This can provide a thought that needs to develop better treatments or improved approaches to address this concern?
Response 5: We have further expanded the drawback of frequent intravitreal injections and addressed the need of more sustained drug delivery system (Lines 162-167).

Comment 6: Line 134: Longer intraocular half-life from DARPins compared with ranibizumab could be addressed (>13 days vs 7.2 days)
Response 6: We have added half-life comparison of DARPins and ranibizumab together with 2 references in the main text (Lines 185-186)

Comment 7: Line 161: Is PLGA-based drug delivery has been approved to use in ophthalmology?
Response 7: The PLGA-based drug, Ozurdex (dexamethasone intravitreal implant), was approved by the FDA in 2009 for use in patients with macular edema following retinal vein occlusion. We have added this information in the main text (Lines 215-217)

Comment 8: Lines 173-179: The last paragraph here is very confusing. It would be good to bring this point up in the front of section and clarify why improving

the durability of anti-VEGF agents requires further attention, but “broaden anti-VEGF activities” is not in the scope of discussion.
Response 8: To minimize confusion, we have removed this paragraph. Instead, we expanded the need of improving the durability of anti-VEGF agents in the beginning of the section II. Please refer to comment 4.

Comment 9: RNA interference has also been classified as a type of gene therapy. The authors should briefly introduce it if the review was emphasizing the application of gene therapy for the treatment of ocular angiogenesis.
Response 9: We have now added a paragraph describing synthetic siRNA (bevasiranib) in the anti-VEGF gene therapy strategy section (Lines 404-412).

Comment 10: Line 321-325: Should mention the variability in sFlt expression was correlated with anti-AAV2 antibodies.
Response 10: We have now included additional information on serum neutralizing antibody against AAV2 and therapeutic gene (sFlt 01) expression in the main text (Lines 364-367).

Comment 11: Line 336: A median 44-week results have been published from the trial website.
Response 11: We have added the 44-week outcome of the OPTIC study (Lines 383-386).

Comment 12: Lines 354: Since the topic focuses on anti-VEGF gene therapy, Retinostat may not well fit into the discussion.
Response 12: We agree that retinostat is not directly relevant to anti-VEGF gene therapy, and have thus removed it from the main text.

Comment 13: Line 388: The large and small size of Cas endonuclease need to be defined here. The gene size of SpCas9/SaCas9/CjCas9 can be mentioned here, this information will be useful to bring up the limitation of payload capacity of the viral vector.
Response 13: We have added additional information on the size of different Cas9 orthologs and mentioned smaller Cas9 variants (SaCas9 and CjCas9) as having better potential for clinical translation as they can be packaged into a single AAV vector (Lines 432-433).

Comment 14: Line 394: A study “Huang et al., Cpf1 Nat Commun,

2017;8(1):112." Should be mentioned in the review to point out the use of two AAV vector CRISPR/Cas system for targeting ocular angiogenesis.
Response 14: We thank the reviewer for recommending additional reference. We believe Huang et al's study utilized SpCas9, not Cpf1. We have summarized and added this reference, and further elaborated on the AAV dual vector system targeting ocular angiogenesis (Lines 450-454).

Comment 15: Line 426: Figure "3" should corrected as "2". A statistical graph or border marquees in Figure 2C should be included to illustrate the suppression of CNV.
Response 15: We have corrected Figure 3 to Figure 2 (Line 477). In addition, border marquees have been added to Figure 2C.

Comment 16: Please make sure gene nomenclature correctly used throughout the manuscript.
Response 16: We have checked and corrected gene nomenclature throughout the manuscript

Comment 17: Please carefully go through the writing of the manuscript, some typos and grammar errors require to be corrected.
Response 17: We have corrected typos and grammatical errors throughout the manuscript.

Comment 18: Please consistent to either use the term "ocular angiogenesis" or "ocular neovascularization" to describe pathological retinal angiogenesis in the manuscript.
Response 18: We have changed "ocular neovascularization" to "ocular angiogenesis" throughout the manuscript.

Comment 19: For information related to clinical trials, please provide the trial number.
Response 19: We have added clinical trial numbers in each trial throughout the manuscript.

Comment 20: Line 20: "CRISPR genome editing" should be corrected as "CRISPR-Cas genome editing"
Response 20: We have corrected "CRISPR genome editing" to CRISPR-Cas technology" (line 60)

Comment 21: Lines 151: Please clarify what was the "laboratory animals"?
Response 21: We have specified the "laboratory animals" as "the rabbit retina" (Line 201)

Comment 22: Lines 169: Please clarify what was the "laboratory animals"?
Response 22: We have changed "laboratory animals" to "rats and mice" (Lines 220).

Comment 23: Line 378: "permanently" is Italic type.
Response 23: We appreciate reviewer's comments, but the Italic type for "permanent" was intentional to emphasize the permanent genome editing effect of CRISPR-Cas system, thus we did not change it.

Comment 24: Line 410: The in-text reference "94" is in a different reference format.
Response 24: The format of reference is now consistent throughout the manuscript.

Comment 25: Line 333: "comparable to animals receiving intravitreal injections of 1.2 mg of aflibercept". Are the "animals" referring non-human primates? What was the frequency of aflibercept injection in the comparison? Please clarify.
Response 25: The study used non-human primates (NHP) only, and compared CNV size between the NHP received intravitreal injection ADVM-022 and 1.2mg of aflibercept. The NHPs that received aflibercept had the treatment only once at the time of CNV induction. We now incorporated this information in the main text (Line 377).

Comment 26: Lines 378-379: "Clustered regularly interspaced short palindromic repeats (CRISPR) systems". Suggest to use "clustered regularly interspaced short palindromic repeats and CRISPR-associated protein (CRISPR-Cas) system" here.
Response 26: We have changed "Clustered regularly interspaced short palindromic repeats (CRISPR) systems" to "clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (CRISPR-Cas) system" in the main manuscript (Lines 418-419).

Comment 27: Lines 381-385: Reference should be provided for "CRISPR-Cas

system".
Response 27: We now have added a reference to the main manuscript (Line 425)

Comment 28: Line 399: Cpf1 should refer as CRISPR-Cas12a
Response 28: We have changed Cpf1 to CRISPR-Cas12a (Line 442).

Reviewer B
Comment 1: Table 2: Although this table is entitled "Anti-angiogenic therapies for increased durability" to content includes approaches to gene therapy. Please confirm this is correct.
Response 1: We appreciate reviewers' comments on the title of Table 2. We agree that the Table was mostly focusing on gene therapy (especially CRISPR studies), and we apologize for our mistake. We now modified the table to summarize all anti-angiogenic therapies for increased durability that are mentioned in this manuscript.

Comment 2: Line 102: Please add that laser treatment leads to permanent damage of the target retina which can be associated with vision loss
Response 2: We have modified and added a sentence "These destructive leaser treatments were designed to halt the disease progression, but can cause permanent damage of target retina and subsequent vision loss." (line 86-87).

Comment 3: Reference 36: reference information --volume/issue number missing
Response 3: We added volume/issue, and page numbers in reference 36 (Line 607).

Comment 4: Line 451: "10" appears to be a typo; consider removing.
Response 4: We appreciate reviewer's comment, however, we believe Leber Congenital Amaurosis 10 is the full name of the disease. To minimize confusion, we have added abbreviation for the disease name, LCA10 (line 437).